JHR 71: 157–161 (2019)
doi: 10.3897/jhr.71.35951
http://jhr.pensoft.net

SHORT COMMUNICATION

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

# Chromosomes of *Eupristina verticillata* Waterston, 1921 and an overview of known karyotypes of chalcid wasps of the family Agaonidae (Hymenoptera)

Vladimir E. Gokhman[1], Francesco Nugnes[2], Umberto Bernardo[2]

**1** *Botanical Garden, Moscow State University, Moscow, Russia* **2** *Institute for Sustainable Plant Protection, Italian National Research Council, Portici (Napoli), Italy*

Corresponding author: *Vladimir E. Gokhman* (vegokhman@hotmail.com)

Academic editor: *Petr Jansta* | Received 6 May 2019 | Accepted 24 July 2019 | Published 30 August 2019

*http://zoobank.org/14EAED77-EE37-480F-AF17-032AC283FF3B*

**Citation:** Gokhman VE, Nugnes F, Bernardo U (2019) Chromosomes of *Eupristina verticillata* Waterston, 1921 and an overview of known karyotypes of chalcid wasps of the family Agaonidae (Hymenoptera). Journal of Hymenoptera Research 71: 157–161. https://doi.org/10.3897/jhr.71.35951

## Abstract

The karyotype of *Eupristina verticillata* Waterston, 1921 (Agaonidae) from Italy was studied for the first time using chromosome morphometrics. The present study showed that this species has n = 6 and 2n = 12, with five larger metacentrics and a smaller acrocentric chromosome in the haploid set. A brief overview of known karyotypes of chalcid wasps of the Agaonidae is given; certain features of karyotype evolution of this family are discussed.

## Keywords

Chalcidoidea, Agaonidae, chromosomes, karyotypes

## Introduction

Chalcidoidea are one of the most speciose, taxonomically complicated and economically important groups of parasitoid Hymenoptera (Godfray 1994, Quicke 1997). With its exceptionally high morphological and ecological diversity, this superfamily includes about 23 thousand known species (Huber 2017), but karyotypes of less than

Copyright *Vladimir E. Gokhman et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

200 members of this group are studied up to now (Gokhman 2013). For example, in the family Agaonidae, a highly specialized tropical/subtropical group of Chalcidoidea exclusively associated with fruits of the plant genus *Ficus*, chromosomes of only six species were previously studied (Gokhman et al. 2010, Liu et al. 2011, Chen et al. 2018). We have recently examined chromosomes of another member of this family, *Eupristina verticillata* Waterston, 1921 collected in Italy about 30 years after its introduction (Lo Verde et al. 1991, 2007). In addition, we briefly overview the current state of knowledge of the karyotypic diversity of Agaonidae.

## Material and methods

### Origin of the material studied

Green syconia of *Ficus microcarpa* Linnaeus, 1782 (Moraceae) containing immature stages of *E. verticillata* were collected by the senior author in July 2018 in the Villa Comunale (city park) of Napoli, Italy (40°49'58.4"N 14°14'3"E). Immediately after collection, the syconia were dissected in the lab, and wasp prepupae were extracted from the galls.

### Preparation of chromosomes

Chromosomal preparations were obtained from cerebral ganglia of prepupae of *E. verticillata* generally following the protocol developed by Imai et al. (1988) with certain modifications. Ganglia were extracted from insects dissected in 0.5% hypotonic sodium citrate solution containing 0.005% colchicine. The extracted ganglia were then transferred to a fresh portion of the hypotonic solution and incubated for 30 min at room temperature. The material was transferred onto a pre-cleaned microscope slide using a Pasteur pipette and then gently flushed with Fixative I (glacial acetic acid: absolute ethanol: distilled water 3:3:4). The tissues were disrupted using dissecting needles in an additional drop of Fixative I. Another drop of Fixative II (glacial acetic acid: absolute ethanol 1:1) was applied to the center of the area, and the more aqueous phase was blotted off the edges of the slide. The slides were then dried for approximately half an hour and stored at room temperature. Chromosome preparations were stained with freshly prepared 3% Giemsa solution in 0.05M Sorensen's phosphate buffer ($Na_2HPO_4$ + $KH_2PO_4$, pH 6.8) for a few hours.

### Image acquisition and analysis

Metaphase plates were analyzed under a Zeiss Axioskop 40 FL epifluorescence microscope (Carl Zeiss, Göttingen, Germany). Images of chromosomes were taken with Zeiss AxioCam MRc digital camera using Zeiss AxioVision software version

3.1. To prepare illustrations, the resulting images were arranged and enhanced with Adobe Photoshop 8.0. KaryoType version 2.0 software was also used for taking measurements from four diploid metaphase plates of *E. verticillata* having well-defined chromosome morphology. For each chromosome of the haploid set, we report both its relative length (100 × length of the chromosome divided by total length of the set) and centromeric index (100 × length of the shorter arm divided by total length of the chromosome). The chromosomes were classified into metacentrics (M), subtelocentrics (ST) and acrocentrics (A) following guidelines provided by Levan et al. (1964).

## Results and discussion

The diploid karyotype of *E. verticillata* contains the largest pair of metacentric chromosomes, two pairs of shorter metacentrics, two other pairs of considerably smaller metacentric chromosomes, and the shortest pair of acrocentrics (Fig. 1; Table 1). On the other hand, its haploid karyotype contains six chromosomes which sizes and shapes are similar to those of the diploid chromosome set (not shown here); the chromosome number in this species is therefore n = 6 and 2n = 12.

The karyotype of *E. verticillata* is structurally similar to that of the only known member of the family Agaonidae with the same chromosome number (Table 2), *Blastophaga psenes* (Gokhman et al. 2010), although chromosome sets of these two species differ in some details. Specifically, diploid karyotypes of both *E. verticillata* and *B. psenes* contain five pairs of metacentrics, but the smallest pairs within their chromosome sets are acrocentric and subtelocentric respectively. In addition, the lengths of most metacentric chromosomes differ substantially in the former species compared to *B. psenes* (see Gokhman et al. 2010). Moreover, the smallest pair of chromosomes is much shorter than the preceding one in the latter species, in contrast to *E. verticillata*. These two chalcid wasps belong to different subfamilies of Agaonidae, Blastophaginae and Agaoninae respectively (Cruaud et al. 2010), and the similar karyotype structure of these species may be considered a plesiomorphic feature of the family in general (Gokhman et al. 2010). Furthermore, chromosome sets of all other studied members of Agaonidae, i.e. two species of the same genus *Eupristina* Saunders, 1882, *E. altissima* and *Eupristina* sp. with n = 5, as well as three members of the genus *Ceratosolen* Mayr, 1885 from the subfamily Kradibiinae, *C. solmsi*, *C. gravelyi*, and *C. emarginatus* with the same chromosome number (Table 2), contain only large metacentrics of similar size (Liu et al. 2011, Chen et al. 2018). Analogously to the situation observed in a few other chalcid families, e.g. Eulophidae, Torymidae and Ormyridae, results of the present study apparently confirm that the karyotypes with n = 5 resulted from the fusion between the smallest acrocentric/subtelocentric chromosome and a particular metacentric in an ancestral chromosome set with n = 6 (Gokhman 2013). If this is true, lower chromosome numbers of corresponding species could represent their putative synapomorphies within certain genera.

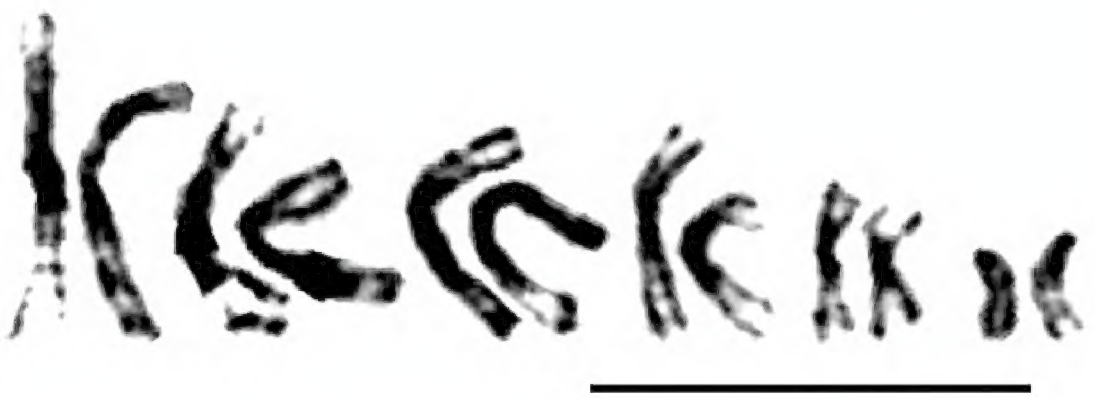

**Figure 1.** Diploid karyotype of *E. verticillata*. Scale bar: 10 μm.

**Table 1.** Relative lengths (RL) and centromeric indices (CI) of chromosomes of *E. verticillata* (mean ± SD).

| Chr. no. | RL | CI |
|---|---|---|
| 1 | 23.30 ± 1.35 | 42.38 ± 3.97 |
| 2 | 21.00 ± 0.77 | 44.53 ± 3.15 |
| 3 | 20.64 ± 0.76 | 43.11 ± 5.28 |
| 4 | 15.63 ± 0.31 | 46.72 ± 2.71 |
| 5 | 11.51 ± 0.78 | 46.21 ± 2.82 |
| 6 | 7.92 ± 0.46 | 0 |

**Table 2.** Characteristics of diploid karyotypes of studied species of Agaonidae.

| Species | 2n | Chromosome formula | Reference |
|---|---|---|---|
| *Blastophaga psenes* (Linnaeus, 1758) | 12 | 10M + 2ST | Gokhman et al. 2010 |
| *Ceratosolen emarginatus* Mayr, 1916 | 10 | 10M | Liu et al. 2011 |
| *C. gravelyi* Grandi, 1916 | 10 | 10M | Liu et al. 2011 |
| *C. solmsi* (Mayr, 1885) | 10 | 10M | Liu et al. 2011 |
| *Eupristina altissima* Balakrishnan & Abdurahiman, 1981 | 10 | 10M | Chen et al. 2018 |
| *E. verticillata* Waterston, 1921 | 12 | 10M + 2A | Present study |
| *Eupristina* sp. | 10 | 10M | Chen et al. 2018 |

## Acknowledgements

The present study was partly supported by a research grant no. 18-04-00611 from the Russian Foundation for Basic Research to VEG.

## References

Chen FM, Zhang XM, Liu Q (2018) Comparison study on the chromosome of two *Eupristina* species from *Ficus altissima* Blume. Journal of Environmental Entomology 40(1): 193–198. [in Chinese with English summary]

Cruaud A, Jabbour-Zahab R, Genson G, Cruaud C, Couloux A, Kjellberg F, van Noort S, Rasplus JY (2010) Laying the foundations for a new classification of Agaonidae (Hymenop-

tera: Chalcidoidea), a multilocus phylogenetic approach. Cladistics 26: 359–387. https://doi.org/10.1111/j.1096-0031.2009.00291.x

Godfray HCJ (1994) Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton, 475 pp.

Gokhman VE (2009) Karyotypes of parasitic Hymenoptera. Dordrecht, Springer, 183 pp. https://doi.org/10.1007/978-1-4020-9807-9

Gokhman VE (2013) Parallel pathways of karyotype evolution in the superfamily Chalcidoidea (Hymenoptera). Russian Entomological Journal 22(3): 177–179.

Gokhman VE, Gumovsky AV (2009) Main trends of karyotype evolution in the superfamily Chalcidoidea (Hymenoptera). Comparative Cytogenetics 3(1): 63–69. https://doi.org/10.3897/compcytogen.v3i1.1

Gokhman VE, Mikhailenko AP, Fursov VN (2010) Chromosomes of *Blastophaga psenes* (Hymenoptera: Agaonidae). Journal of Hymenoptera Research 19(1): 187–188.

Huber JT (2017) Biodiversity of Hymenoptera. In: Foottit RG, Adler PH (Eds) Insect biodiversity: science and society (2nd edn). Wiley Blackwell, Oxford, 419–461. https://doi.org/10.1002/9781118945568.ch12

Imai HT, Taylor RW, Crosland MWJ, Crozier RH (1988) Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis. Japanese Journal of Genetics 63: 159–185. https://doi.org/10.1266/jjg.63.159

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

Lo Verde G, Porcelli F, Sinacori A (1991) Presenza di *Parapristina verticillata* (Waterst.) e *Odontofroggatia galili* Wiebes (Hymenoptera: Chalcidoidea Agaonidae) in Sicilia. Atti del XVI Congresso Nazionale Italiano di Entomologia, 139–143.

Lo Verde G, Porcelli F, Bella S, Rasplus JI (2007) Imenotteri Agaonidi nuovi per l'Europa e loro ruolo nella naturalizzazione di *Ficus* spp. in Italia. Atti del XXI Congresso Nazionale Italiano di Entomologia, 60.

Liu Q, Ou Xh, Compton SG, Yang Dr (2011) Chromosome numbers are not fixed in Agaonidae (Hymenoptera: Chalcidoidea). Symbiosis 53: 131–137. https://doi.org/10.1007/s13199-011-0116-4

Quicke DLJ (1997) Parasitic wasps. London, Chapman & Hall, 470 pp.